EXTRACTION OF 3-HYDROXYALKANOIC ACID

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Abstract of **JP2001057895**

PROBLEM TO BE SOLVED: To efficiently extract and separate the subject compound by adding a divalent or polyvalent metal salt and a surfactant to a suspension of a microbial cell of a poly-3-hydroxyalkanoic acid-containing microorganism in an extraction solvent and flocculating and removing an undissolved cell residue. SOLUTION: A divalent or polyvalent metal salt (e.g. calcium chloride, etc.), and/or a surfactant (e.g. benzyltrimethlammonium chloride, etc.), is added to a suspension of a microbial cell of poly-3-hydroxyalkanoic acid (PHA)-containing microorganism [e.g. Aicaligenes eutrophus A32C(FERM P-15786) strain into which a PHA synthase gene derived from Aeromonas caviae is transferred, etc.], and an extraction solvent (e.g. chloroform, etc.), and undissolved cell residue is flocculated and removed from the PHA-containing solution to readily obtain a high-purity poly-3-hydroxyalkanoic acid useful as a biodegradable plastic, etc., in an improved efficiency of industrial production at a low cost.

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(54)【発明の名称】　ポリ－３－ヒドロキシアルカン酸の抽出方法

(57)【要約】
【課題】　ＰＨＡを含有する微生物菌体からの、ＰＨＡの抽出分離を行うための抽出方法を提供すること。
【解決手段】　ＰＨＡを含有する微生物菌体と抽出溶媒との懸濁液に、金属塩およびまたは界面活性剤を添加して、未溶解細胞残査を凝集させて除去することによって、効率よくＰＨＡ溶液を分離することを特徴とするＰＨＡの抽出方法。

【００１１】別の好ましい実施態様としては、ＰＨＡを含有する微生物が、アエロモナス・キャビエ由来のＰＨＡ合成酵素群遺伝子が導入された菌株である上記抽出分離方法に関する。

【００１２】更に別の好ましい実施態様としては、ＰＨＡが、３ＨＢと３ＨＨとの２成分共重合体、または、３ＨＢと３ＨＶと３ＨＨとの３成分共重合体である上記抽出分離方法に関する。

【００１３】

【発明の実施の形態】本発明に用いる微生物は、細胞内にＰＨＡを蓄積している微生物であれば特に限定されない。例えば、アルカリゲネス・リポリチカ（Ａｉｃａｌｉｇｅｎｅｓｌｉｐｏｌｙｔｉｃａ）、アルカリゲネス・ユウトロファス（Ａｉｃａｌｉｇｅｎｅｓ　ｅｕｔｒｏｐｈｕｓ）、アルカリゲネス・ラタス（Ａｉｃａｌｉｇｅｎｅｓ　ｌａｔａｓ）等のアルカリゲネス属（Ａｌｃａｌｉｇｅｎｅｓ）、シュウドモナス属（Ｐｓｅｕｄｏｍｏｎａｓ）、バチルス属（Ｂａｃｉｌｌｕｓ）、アゾトバクター属（Ａｚｏｔｏｂａｃｔｅｒ）、ノカルディア属（Ｎｏｃａｒｄｉａ）、アエロモナス属（Ａｅｒｏｍｏｎａｓ）の菌が挙げられ、中でも、アエロモナス・キャビエ（Ａｅｒｏｍｏｎａｓ　ｃａｖｉａｅ）等の菌株、または、アエロモナス・キャビエ由来のＰＨＡ合成酵素群の遺伝子が導入された菌株、例えば、アルカリゲネス・ユウトロファスＡ３２Ｃ（寄託番号ＦＥＲＭ Ｐ－１５７８６）等がより好ましい。

【００１４】これらの微生物の培養方法は、ＰＨＡを多量に効率よく菌体内に蓄積できるものであれば特に限定はなく、例えば、前記アルカリゲネス・ユウトロファスＡ３２Ｃ（ＦＥＲＭ Ｐ－１５７８６）を用いる場合には、Ｊ．Ｂａｃｔｅｒｉｏｌ．，１７９，４８２１－４８８０頁（１９９７）等に記載の方法が好ましい。

【００１５】本発明におけるポリ－３－ヒドロキシアルカン酸（ＰＨＡ）とは、特に限定されないが、Ｄ－３－ヒドロキシブチレート（３ＨＢ）のホモポリマーや３ＨＢと他の３－ヒドロキシアルカン酸との共重合体が好ましく、更には、３ＨＢとＤ－３－ヒドロキシヘキサノエート（３ＨＨ）との２成分共重合体（Ｍａｃｒｏｍｏｌｅｃｕｌｅｓ，２８，４８２２－４８２８（１９９５））または、３ＨＢとＤ－３－ヒドロキシバレレート（３ＨＶ）と３ＨＨとの３成分共重合体（特開平０８－２８９７９７号）などが、物性の面からより好ましい。ここで、３ＨＢと３ＨＨの２成分共重合体を構成する各モノマーユニットの組成比については特に限定されるものではないが、３ＨＢユニットの含有量が１～９９モル％といった組成比のものが好適である。また、３ＨＢと３ＨＶと３ＨＨとの３成分共重合体を構成する各モノマーユニットの組成比については特に限定されるものではないが、例えば、３ＨＢユニット含有量が１～９５モル％、３ＨＶユニット含有量が１～９６モル％、３ＨＨユニット含有量が１～３０モル％といった組成比のものが好適である。またこれらＰＨＡの分子量は１０万以上が好ましく、５０万以上がより好ましい。

【００１６】ＰＨＡの微生物菌体中の含有率は、高い方が好ましいのは当然であり、工業レベルでの適用においては乾燥菌体中に２０重量％以上が好ましく、抽出操作、分離操作、分離ポリマーの純度等を考慮すると５０重量％以上が特に好ましい。本発明においては、前記のようにして培養して得られた微生物菌体を、培養液から分離した湿菌体としてそのまま用いても良いし、または湿菌体を凍結乾燥機等で乾燥処理して乾燥菌体として用いても良い。さらには、ミルや高圧ホモジナイザー等の物理的破砕処理、界面活性剤、次亜塩素酸ナトリウムや有機溶剤等の化学処理で菌体の一部を破壊し、または菌体の一部を除去してＰＨＡの含有量を高めたものを用いても良い。

【００１７】本発明で使用するＰＨＡの抽出溶媒としては、ＰＨＡが溶解するものであれば特に限定されず、例えば、クロロホルム、塩化メチレン、１，２－ジクロロエタン、ピリジン、１，２－プロピレンカーボネートのような環式カーボネート類、テトラヒドロフラン、乳酸エチルやアセトニトリル等やこれらの溶媒の混合物、例えばクロロホルムとメタノールの混合物やクロロホルムとテトラヒドロフランの混合物等の混合溶媒系が挙げられる。

【００１８】本発明で使用する金属塩としては、２価以上の金属イオンと、一般的な対イオンからなる金属塩であれば特に限定されず、例えば、金属イオンとしては、カルシウム、マグネシウム、鉄、亜鉛、アルミニウム、バリウム、マンガン、銅、コバルト等が挙げられ、対イオンとしては、塩化物イオン、硫酸イオン、リン酸イオン、硝酸イオン、炭酸イオン等が挙げられ、金属塩の具体的な例としては、塩化カルシウム、塩化マグネシウム、塩化第一鉄、塩化第二鉄、塩化亜鉛、塩化バリウム、塩化コバルト、塩化銅、塩化マンガン、塩化アルミニウム、硫酸マグネシウム、硫酸亜鉛、炭酸カルシウム、炭酸マグネシウム等が例示できる。また、本発明で使用される界面活性剤としては、陰イオン性、陽イオン性、両性もしくは非イオン性でも良いが、好ましくは陽イオン性界面活性剤であり、具体的には、セチルトリメチルアンモニウムブロミド、ドデシルピリジニウムクロリド、テトラデシルアンモニウムブロミド、セチルピリジニウムクロリド、トリエチルヘキシルアンモニウムブロミド、４，４－トリメチレンビス（１－メチルピペリヂン）、トリメチルフェニルアンモニウムブロミド、ベンジルトリメチルアンモニウムクロリド、ヘキサデシルトリメチルアンモニウムブロミド、アセタミン８６（花王株式会社製）コータミン２４Ｐ（花王株式会社製）等が挙げられる。

【００１９】本発明で使用する金属塩や界面活性剤の添

ァス（ＡＴＣＣ１７６９９）株を、グルコースを炭素源として培養し（培地：グルコース　20g、$Na_2HPO_4 \cdot 12H_2O$ 9g、KH_2PO_4 1.5g、$(NH_4)_2SO_4$ 6g、$MgSO_4 \cdot 7H_2O$ 0.2g、微量金属元素溶液（組成：$FeCl_2 \cdot 6H_2O$ 16.2g、$CaCl_2 \cdot 2H_2O$ 10.3g、$CoCl_2 \cdot 6H_2O$ 0.2g、$NiCl_3 \cdot 6H_2O$ 0.1g、$CrCl_3 \cdot 6H_2O$ 16.2g、$CuSO_4 \cdot 5H_2O$ 0.2g / 1L 0.1N-HCl）5ml / 1L、ｐＨ６．８、培養温度30℃、培養時間４８時間）、３ＨＢのホモポリマー（３ＨＢユニット　100%）を菌体内に約６０重量%含有した菌体を得た。これを遠心分離処理（５０００ｒｐｍ、１０ｍｉｎ）して培養液から分離し、湿菌体とした。この湿菌体を凍結乾燥し、乾燥菌体としたのちに、乾燥菌体で５０ｇ／ｌとなるようにクロロホルムに懸濁し、室温で５時間撹拌を行って３ＨＢホモポリマーの抽出を行った。この微生物菌体を含む抽出液に、陽イオン性界面活性剤であるベンジルトリメチルアンモニウムクロリドを１０ｇ／Ｉとなるように加えて更に１時間撹拌しクロロホルムに溶解しない細胞残査を凝集させ、これをろ紙（桐山製作所製、Ｎｏ．４）を用いて桐山ロートにて吸引ろ過し、凝集菌体残査を分離除去した。この時目詰まりすることなく、ろ過を行うことが出来た。得られた濾液に、撹拌しながらメタノールを加えて３ＨＢホモポリマーの結晶を析出させ、該結晶をろ過により集め減圧下に乾燥した。得られた３ＨＢホモポリマーの回収率を計算したところ、９５%であった。

【００３０】（実施例７）実施例６において、陽イオン性界面活性剤であるベンジルトリメチルアンモニウムクロリドをヘキサデシルトリメチルアンモニウムブロミドに変更した以外は同様の操作を行った。得られた３ＨＢホモポリマーの回収率は９４%であった。

【００３１】（実施例８）実施例６において、抽出溶媒をクロロホルムからテトラヒドロフランに変更した以外は同様の操作を行った。得られた３ＨＢホモポリマーの回収率は８５%であった。

【００３２】（実施例９）実施例６で得られた湿菌体を、乾燥することなく５０ｇ／ｌとなるようにテトラヒドロフランに懸濁し、加熱還流下で５時間撹拌を行って３ＨＢホモポリマーの抽出を行った。この微生物菌体を含む抽出液に、塩化カルシウムを１０ｇ／ｌとなるように加えて更に１時間撹拌し未溶解細胞残査を凝集させ、これをろ紙（桐山製作所製、Ｎｏ．４）を用いて桐山ロートにて吸引ろ過し、凝集菌体残査を分離除去した。この時目詰まりすることなくろ過を行うことが出来た。得られた濾液を、撹拌しながら室温まで冷却し、３ＨＢホモポリマーの結晶を析出させ、該結晶をろ過により集め減圧下に乾燥した。得られた３ＨＢホモポリマーの回収率は８１%であった。

【００３３】（実施例１０）実施例９において、塩化カルシウムを陽イオン性界面活性剤であるベンジルトリメチルアンモニウムクロリドに変更した以外は同様の操作を行った。得られた３ＨＢホモポリマーの回収率は８３%であった。

【００３４】（実施例１１）実施例１において、アルカリゲネス・ユウトロファス　ＡＣ３２（ＦＥＲＭＰ－１５７８６）をアエロモナス・キャビエ　ＦＡ４４０（寄託番号ＦＥＲＭＢＰ－３４３２）に変更した以外は同様の条件で培養し、３ＨＢと３ＨＨとの２成分共重合体（３ＨＢユニット：３ＨＨユニット＝１０：９０（モル比））を約３０重量%含有した菌体を得た。これを遠心分離処理（５０００ｒｐｍ、１０ｍｉｎ）して培養液から分離し、湿菌体とした。この湿菌体を凍結乾燥し、乾燥菌体としたのちに、乾燥菌体で５０ｇ／ｌとなるようにクロロホルムに懸濁し、室温で５時間撹拌を行って３ＨＢと３ＨＨとの２成分共重合体の抽出を行った。この微生物菌体を含む抽出液に、陽イオン界面活性剤であるベンジルトリメチルアンモニウムクロリドを１０ｇ／ｌとなるように加えて更に１時間撹拌し未溶解細胞残査を凝集させ、これをろ紙（桐山製作所製、Ｎｏ．４）を用いて桐山ロートにて吸引ろ過し、凝集菌体残査を分離除去した。この時目詰まりすることなくろ過を行うことが出来た。得られた濾液に、撹拌しながらメタノールを加えて３ＨＢと３ＨＨとの２成分共重合体の結晶を析出させ、該結晶をろ過により集め減圧下に乾燥した。得られた３ＨＢと３ＨＨとの２成分共重合体の回収率を計算したところ、９６%であった。

【００３５】（比較例１）実施例１において、ベンジルトリメチルアンモニウムクロライドを添加しなかった以外は同様の操作を行った。ろ過の段階で目詰まりが激しく菌体残渣を分離することができなかった。

【００３６】（比較例２）実施例６において、ベンジルトリメチルアンモニウムクロリドを添加しなかった以外は同様の操作を行った。その結果、ろ過の段階で目詰まりが激しく菌体残渣を分離することができなかった。

【００３７】

【発明の効果】本発明によれば、ＰＨＡを含有する微生物菌体と抽出溶媒との懸濁液に、２価以上の金属塩や界面活性剤を添加するという極めて簡便な操作によって、未溶解細胞残査を凝集させて除去することが可能となり、容易に高純度のＰＨＡが得られるため、本発明は、微生物によるＰＨＡの工業的生産の効率向上およびコストの低減に大きく寄与するものである。

PATENT ABSTRACTS OF JAPAN

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(54) EXTRACTION OF 3-HYDROXYALKANOIC ACID

(57)Abstract:
PROBLEM TO BE SOLVED: To efficiently extract and separate the subject compound by adding a divalent or polyvalent metal salt and a surfactant to a suspension of a microbial cell of a poly-3-hydroxyalkanoic acid-containing microorganism in an extraction solvent and flocculating and removing an undissolved cell residue.
SOLUTION: A divalent or polyvalent metal salt (e.g. calcium chloride, etc.), and/or a surfactant (e.g. benzyltrimethlammonium chloride, etc.), is added to a suspension of a microbial cell of poly-3-hydroxyalkanoic acid (PHA)-containing microorganism [e.g. Aicaligenes eutrophus A32C (FERM P-15786) strain into which a PHA synthase gene derived from Aeromonas caviae is transferred, etc.], and an extraction solvent (e.g. chloroform, etc.), and undissolved cell residue is flocculated and removed from the PHA-containing solution to readily obtain a high-purity poly-3-hydroxyalkanoic acid useful as a biodegradable plastic, etc., in an improved efficiency of industrial production at a low cost.

LEGAL STATUS

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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] The extraction separation approach of the Polly 3-hydroxy alkane acid characterized by adding the metal salt and/or surfactant more than divalent, making non-dissolved cell residue condense to the suspension of the microorganism biomass containing a Polly 3-hydroxy alkane acid, and an extracting solvent, and removing from the solution containing a Polly 3-hydroxy alkane acid to it.
[Claim 2] The extraction separation approach of a Polly 3-hydroxy alkane acid according to claim 1 that a surfactant is cation nature.
[Claim 3] The extraction separation approach according to claim 1 or 2 that the microorganism containing a Polly 3-hydroxy alkane acid is the strain into which the Polly 3-hydroxy alkane acid synthetic enzyme group gene of the Aeromonas KYABIE origin was introduced.
[Claim 4] The extraction separation approach according to claim 1 to 3 that a Polly 3-hydroxy alkane acid is 3 component copolymer of 2 component copolymer of D-3-hydroxy butyrate (3HB) and D-3-hydroxy hexanoate (3HH) or D-3-hydroxy butyrate (3HB) and D-3-hydroxyvalerate (3HV), and D-3-hydroxy hexanoate (3HH).

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the extraction separation approach of a Polly 3-hydroxy alkane acid from a microorganism biomass.
[0002]
[Description of the Prior Art] Although current and a plastic waste are processed by incineration, reclamation, etc., there are troubles, such as warming of the earth and ground relaxation of a reclaimed ground, in these arts. Therefore, recycle system-ization is progressing with a rise of the social consciousness to plastics recycle. However, there is much what remains there being a limitation in a recyclable application, could not respond only by incineration, reclamation, and recycle as a plastics abolition art as a practical question, and left in a nature. Then, after abolition, it is incorporated by the cyclical change of materials of a nature, the biodegradable plastic from which a decomposition product does not become harmful attracts attention, and it is anxious for the utilization.
[0003] Also in these biodegradation plastics, a Polly 3-hydroxy alkane acid (PHA is called henceforth) is thermoplastic polyester which has the biodegradability which is generated and is accumulated as energy are recording matter into the biomass of many microorganism kinds, and since it is incorporated by the carbon cycle process of a nature, and it is expected that there is almost no adverse effect to an ecosystem, it attracts attention especially. Moreover, also in the medical field, it is thought that the implant material of recovery needlessness and the utilization as a drug carrier are possible.
[0004] PHA generated by the microorganism forms the microsome and is accumulated into the biomass, and in order to use these as plastics, it is necessary to separate and take it out from the inside of the biomass of a microorganism. As a known approach of carrying out separation purification of the PHA from a microorganism biomass, when it divides roughly, there are an approach which PHA makes dissolve PHA in a meltable organic solvent, and extracts, and a method of obtaining PHA by making biomass constituents other than PHA solubilize, and removing.
[0005] As the extraction separation approach of PHA using an organic solvent, the approach using the extracting solvent of a hydrophilic property like the approach (JP,55-118394,A, JP,57-65193,A) using hydrophobic halogen content hydrocarbons, such as 1,2-dichloroethane and chloroform, as an extracting solvent and dioxane (JP,63-198991,A), a propanediol (JP,02-69187,A), or a tetrahydrofuran (JP,07-79788,A) is proposed, for example. However, if it is going to dissolve PHA in these approaches to the concentration which deserves practical use, the extract serves as **** extremely and has the fault that separation with the biomass residue which was not dissolved in an extracting solvent and the solvent layer containing PHA is very difficult.
[0006] although some methods of obtaining PHA by making biomass constituents other than PHA solubilize, and on the other hand removing are also proposed (J. — Gen. Microbiology — 19,198 - 209 pages (1958)) JP,04-61638,B, Patent Publication Heisei No. 502415 [08 to], JP,07-177894,A, The actual condition is the approaches neither is suitable for practical use —

the remarkable depolymerize of PHA happens, or have troubles, like the purity of PHA obtained is low upwards, and down stream processing is mostly complicated, or need a toxic high chemical or cost becomes very high.
[0007]
[Problem(s) to be Solved by the Invention] The object of this invention is in the extract of PHA from a microorganism biomass to offer the approach of separating efficiently the biomass residue which was not dissolved in an extracting solvent, and the solvent layer containing PHA.
[0008]
[Means for Solving the Problem] As a result of examining wholeheartedly how PHA is producible industrially advantageous, this invention persons made the cell residue which was not dissolved in the suspension of the microorganism biomass containing PHA, and an extracting solvent by adding the metal salt more than divalent, or a surfactant at an extract condense, found out that separation clearance could be carried out efficiently, and reached this invention.
[0009] That is, this invention relates to the extraction separation approach of PHA characterized by adding the metal salt and/or surfactant more than divalent, making non-dissolved cell residue condense to the suspension of the microorganism biomass containing PHA, and an extracting solvent, and removing from the solution containing PHA to it.
[0010] As a desirable embodiment, a surfactant is related with the above-mentioned extraction separation approach which is cation nature.
[0011] As another desirable embodiment, the microorganism containing PHA is related with the above-mentioned extraction separation approach which is strain that the PHA synthetic enzyme group gene of the Aeromonas KYABIE origin was introduced.
[0012] Furthermore, as another desirable embodiment, PHA is related with the above-mentioned extraction separation approach which is 2 component copolymer of 3HB and 3HH(s), or 3 component copolymer of 3HB, 3HV, and 3HH(s).
[0013]
[Embodiment of the Invention] The microorganism used for this invention will not be limited especially if it is the microorganism which is accumulating PHA in intracellular. For example, Alcaligenes RIPORICHIKA (Alcaligeneslipolytica), Alcaligenes eutrophus (Alcaligenes eutrophus), Alcaligenes, such as Alcaligenes RATASU (Alcaligenes latus) (Alcaligenes), Pseudomonas (Pseudomonas), Bacillus (Bacillus), An azotobacter group (Azotobacter), a Nocardia group (Nocardia), The bacillus of Aeromonas (Aeromonas) is mentioned. Especially Strain, such as aloe MONASU KYABIE (Aeromonas caviae), Or the strain into which the gene of the PHA synthetic enzyme group of the Aeromonas KYABIE origin was introduced, for example, Alcaligenes eutrophus A32C etc., (deposition number FERM P-15786) is more desirable.
[0014] If the culture approach of these microorganisms can accumulate PHA into a biomass efficiently so much, when there is nothing, for example, it uses said Alcaligenes eutrophus A32C (FERM P-15786), the approach given in J.Bacteriol., 179, 4821 - 4830 etc. pages (1997), etc. of especially definition is desirable.
[0015] With the Polly 3-hydroxy alkane acid (PHA) in this invention Although not limited especially, the homopolymer of D-3-hydroxy butyrate (3HB) and the copolymer of 3HB and other 3-hydroxy alkane acids are desirable. Further 2 component copolymer of 3HB and D-3-hydroxy hexanoate (3HH) (Macromolecules, 28, 4822-4828 (1995)) Or 3 component copolymer (JP,08-289797,A) of 3HB, D-3-hydroxyvalerate (3HV), and 3HH(s) etc. is more desirable from the field of physical properties. Although not limited here especially about the presentation ratio of each monomer unit which constitutes 3HB and 2 component copolymer of 3HH(s), the thing of the presentation ratio of 1-99-mol % in the content of 3HB unit is suitable. Moreover, although not limited especially about the presentation ratio of each monomer unit which constitutes 3 component copolymer of 3HB, 3HV, and 3HH(s), the thing of the presentation ratio of [for example, / content / 3HB unit / content / 1-95 mol % and 3HV unit] 1-30-mol % in a 1-96-mol % and 3HH unit content is suitable. Moreover, as for the molecular weight of these PHAs, 100,000 or more are desirable, and 500,000 or more are more desirable.
[0016] the higher one of the content in the microorganism biomass of PHA is desirable — naturally — coming out — it is — application on industrial level — setting — a desiccation

bacillus — when 20 % of the weight or more is inside of the body desirable and the purity of extract operation, separation actuation, and a separation polymer etc. is taken into consideration inside of the body, especially 50 % of the weight or more is desirable. In this invention, the microorganism biomass which is the above, and was cultivated [was made and] and obtained may be used as it is as a wet fungus body separated from culture medium, or with a freeze dryer etc., desiccation processing may be carried out and a wet fungus body may be used as a dried cell. Furthermore, what destroyed a part of biomass by chemical treatments, such as physical crushing processing of a mill, a high voltage homogenizer, etc., a surfactant, and a sodium hypochlorite, an organic solvent, or removed a part of biomass, and raised the content of PHA may be used.
[0017] As an extracting solvent of PHA used by this invention, especially if PHA dissolves, it will not be limited, for example, mixed solvent systems, such as the mixture of chloroform, a methylene chloride, 1,2-dichloroethane, a pyridine, 1, the ring type carbonate like 2-propylene carbonate, a tetrahydrofuran, ethyl lactate, acetonitriles, etc. and these solvents, for example, the mixture of chloroform and a methanol, the mixture of chloroform and a tetrahydrofuran, etc., are mentioned.
[0018] It will not be limited especially if it is the metal salt which consists of a metal ion more than divalent, and a common counter ion as a metal salt used by this invention. As a metal ion Calcium, magnesium, iron, zinc, aluminum, barium, manganese, copper, cobalt, etc. are mentioned. As a counter ion Chloride ion, sulfate ion, phosphoric-acid ion, nitrate ion, carbonate ion, etc. are mentioned. As a concrete example of a metal salt A calcium chloride, a magnesium chloride, ferrous chloride, a ferric chloride, a zinc chloride, barium chloride, a cobalt chloride, a copper chloride, a manganese chloride, an aluminum chloride, magnesium sulfate, a zinc sulfate, a calcium carbonate, a magnesium carbonate, etc. can be illustrated. moreover, as a surfactant used by this invention Although anion nature, cation nature, both sexes, or nonionic are sufficient, it is a cationic surfactant preferably. Specifically Cetyl trimethylammonium bromide, dodecyl pyridinium chloride, Tetradecyl ammonium bromide, cetyl pyridinium chroride, Triethyl hexyl ammonium bromide, 4, and 4-trimethylene screw (1-methyl piperidine), Trimethyl phenyl ammonium bromide, benzyl trimethylammonium chloride, hexadecyl trimethylammonium bromide, ASETAMIN 86 (Kao Corp. make) Kohtamin 24P (Kao Corp. make), etc. are mentioned.
[0019] Although especially the addition of the metal salt used by this invention or a surfactant is not restricted, it is desirable to add so that it may become the concentration of the range of 0.001 - 10 % of the weight of microorganism biomass suspension 1L hits, and the concentration of 0.01 - 5% of the weight of the range is more more desirable still. In the case of the concentration which effectiveness is low and exceeds 10 % of the weight by 0.001 or less % of the weight of concentration, it is not desirable from the field of cost.
[0020] In this invention, someday, it may be independent in or, and the above-mentioned metal salt and a surfactant may be used, and may be used together. It may supply to biomass suspension and may be made to dissolve in it with a liquid or a solid-state, and after using the charge approach of a metal salt or a surfactant as a solution beforehand, it may be supplied to biomass suspension. It is more desirable to stir biomass suspension, in order to promote distribution of the metal salt within biomass suspension or a surfactant on the occasion of the charge of a metal salt and a surfactant. About the mixing time for making the non-dissolved cell residue of a microorganism biomass condense, and stirring temperature, it can set up suitably.
[0021] In this invention, it is carrying out addition processing of a metal salt or the surfactant as mentioned above, and since the non-dissolved cell residue in suspension condenses, a PHA solution is easily separable into the suspension of the microorganism biomass and extracting solvent containing PHA. Although especially the separation actuation that can be used here is not limited, it can use the approach generally learned in filtration, a decantation, a centrifugal separator, membrane separation, etc., for example. About the separation actuation by filtration, a

just to collect the solutions in which the suspension upper part became clear by the suitable approach, for example, an attraction machine etc. the conditions generally known about the separation actuation with a centrifugal separation machine — it can use — a centrifugal separation machine — a batch process and continuous system — either can be used.
[0022] Thus, the polymer purity of the PHA solution obtained by dissociating with non-dissolved cell residue is dramatically high, and if a solvent is removed for this by the well-known approach, PHA of a high grade can be obtained. Of course according to the object, purity can also be further raised using crystallization or the other purification approaches.
[0023]
[Example] Although an example explains this invention below, this invention is not limited to these examples.
[0024] (Example 1) Alcaligenes eutrophus which introduced the PHA synthetic enzyme group gene of the Aeromonas KYABIE origin AC32 (deposition number FERM P-15786) stock J. It cultivates by the approach of a publication in Bacteriol., 179, and 4821 -4830 term (1997) (culture medium: 4.12H2O11.3 g Na2HPO). KH2PO4 1.9g, 2(NH4) SO4 5g, a pro extract (product made from **** Seasoning 10g) MgSO4,7H2O 1g, palm oil 50g, minute amount metallic element solution (presentation: 2.6H2O16.2 g FeCl) CaCl2.2H2O 10.3g, CoCl2.6H2O 0.2g, NiCl3.6H2O 0.1g, CrCl3.6H2O 15.2g, CuSO4 and 5H2O 0.2g / 1L0.1 N-HCl5ml / 1L, The biomass which contained 2 component copolymer (3HB unit: 3HH unit =90:10 (mole ratio), molecular weight about 1 million) of 3HB and 3HH(s) about 50% of the weight was obtained for pH8.7, the culture tamperature of 30 degrees C, and culture time amount 72 hours. Centrifugal separation processing (5000rpm, 10min) of this was carried out, and it dissociated from culture medium and considered as the wet fungus body. After freeze-drying this wet fungus body and considering as a dried cell, it suspended with chloroform so that it might become in l. and 50g /by the dried cell, and stirring was performed at the room temperature for 5 hours, and 2 component copolymer of 3HB and 3HH(s) was extracted. to the extract containing this microorganism biomass, in addition, the benzyl trimethylammonium chloride which is a cationic surfactant is stirred for further 1 hour so that it may become 10 g/l, and non-dissolved cell residue is condensed to it — making — this — a filter paper (made in the Kiriyama factory, No.4) — using — Kiriyama — attraction filtration was carried out with the funnel and separation clearance of the condensation biomass residue was carried out. It was able to filter without carrying out blinding at this time. Stirring, in the obtained filtrate, the methanol was added, the crystal of 2 component copolymer of 3HB and 3HH(s) was deposited, this crystal was brought together by filtration in it, and it dried under reduced pressure to it. It was 98% when the recovery of 2 component copolymer of the obtained 3HB and 3HH(s) was calculated.
[0025] (Example 2) In the example 1, same actuation was performed except having changed into hexadecyl trimethylammonium bromide the benzyl trimethylammonium chloride which is a cationic surfactant. The recovery of 2 component copolymer of the obtained 3HB and 3HH(s) was 95%.
[0026] (Example 3) In the example 1, same actuation was performed except having changed the extracting solvent into the tetrahydrofuran from chloroform. The recovery of 2 component copolymer of the obtained 3HB and 3HH(s) was 87%.
[0027] (Example 4) Without drying the wet fungus body obtained in the example 1, it suspended in the tetrahydrofuran so that it might become in l. and 50g /, and stirring was performed under heating reflux for 5 hours, and 2 component copolymer of 3HB and 3HH(s) was extracted. to the extract containing this microorganism biomass, in addition, a calcium chloride is stirred for further 1 hour so that it may become 10 g/l, and non-dissolved cell residue is condensed to it — making — this — a filter paper (made in the Kiriyama factory, No.4) — using — Kiriyama — attraction filtration was carried out with the funnel and separation clearance of the condensation biomass residue was carried out. It was able to filter without carrying out blinding at this time.

[0028] (Example 5) In the example 4, same actuation was performed except having changed the calcium chloride into benzyl trimethylammonium chloride. The recovery of 2 component copolymer of the obtained 3HB and 3HH(s) was 83%.
[0029] (Example 6) The Alcaligenes eutrophus (ATCC17699) stock A glucose is cultivated as a carbon source (culture medium: glucose 20g and 4.12H2O9 g Na2HPO). KH2PO4 1.5g, 2(NH4) SO4 6g, MgSO4.7H2O 0.2g, Minute amount metallic element solution (presentation: FeCl2.6H2O 16.2g and 2.2H2O10.3 g CaCl) CoCl2.6H2O 0.2g, NiCl3.6H2O 0.1g, CrCl3.6H2O 16.2g, CuSO4 and 5H2O 0.2g / 1L 0.1 N-HCl5ml / 1L, The biomass which contained the 3HB homopolymer (3HB unit 100%) about 60% of the weight in the biomass was obtained for pH6.8, the culture temperature of 30 degrees C, and culture time amount 48 hours. Centrifugal separation processing (5000rpm, 10min) of this was carried out, and it dissociated from culture medium and considered as the wet fungus body. After freeze-drying this wet fungus body and considering as a dried cell, it suspended with chloroform so that it might become 50 g/l by the dried cell, and stirring was performed at the room temperature for 5 hours, and 3HB homopolymer was extracted. the cell residue which, in addition, stirs the benzyl trimethylammonium chloride which is a cationic surfactant to the extract containing this microorganism biomass for further 1 hour so that it may become 10 g/l, and is not dissolved in chloroform at it is condensed — making — this — a filter paper (made in the Kiriyama factory, No.4) — using — Kiriyama — attraction filtration was carried out with the funnel and separation clearance of the condensation biomass residue was carried out. It was able to filter without carrying out blinding at this time. Stirring, in the obtained filtrate, the methanol was added, the crystal of 3HB homopolymer was deposited, this crystal was brought together by filtration in it, and it dried under reduced pressure to it. It was 95% when the recovery of obtained 3HB homopolymer was calculated.
[0030] (Example 7) In the example 6, same actuation was performed except having changed into hexadecyl trimethylammonium bromide the benzyl trimethylammonium chloride which is a cationic surfactant. The recovery of obtained 3HB homopolymer was 94%.
[0031] (Example 8) In the example 6, same actuation was performed except having changed the extracting solvent into the tetrahydrofuran from chloroform. The recovery of obtained 3HB homopolymer was 85%.
[0032] (Example 9) Without drying the wet fungus body obtained in the example 6, it suspended in the tetrahydrofuran so that it might become 50 g/l, and stirring was performed under heating reflux for 5 hours, and 3HB homopolymer was extracted. to the extract containing this microorganism biomass, in addition, a calcium chloride is stirred for further 1 hour so that it may become 10 g/l, and non-dissolved cell residue is condensed to it — making — this — a filter paper (made in the Kiriyama factory, No.4) — using — Kiriyama — attraction filtration was carried out with the funnel and separation clearance of the condensation biomass residue was carried out. It was able to filter without carrying out blinding at this time. The obtained filtrate was cooled to the room temperature, stirring, the crystal of 3HB homopolymer was deposited, these crystals were collected by filtration, and it dried under reduced pressure. The recovery of obtained 3HB homopolymer was 81%.
[0033] (Example 10) In the example 9, same actuation was performed except having changed the calcium chloride into the benzyl trimethylammonium chloride which is a cationic surfactant. The recovery of obtained 3HB homopolymer was 83%.
[0034] (Example 11) It sets in the example 1 and is Alcaligenes eutrophus. It is Aeromonas KYABIE about AC32 (FERMP-15786). Except having changed into FA440 (deposition number FERMBP-3432), it cultivated on the same conditions and the biomass which contained 2 component copolymer (3HB unit: 3HH unit =10:90 (mole ratio)) of 3HB and 3HH(s) about 30% of the weight was obtained. Centrifugal separation processing (5000rpm, 10min) of this was carried out, and it dissociated from culture medium and considered as the wet fungus body. After freeze-drying this wet fungus body and considering as a dried cell, it suspended with chloroform so that it might become in l. and 50g /by the dried cell, and stirring was performed at the room temperature for 5 hours, and 2 component copolymer of 3HB and 3HH(s) was extracted. to the extract containing this microorganism biomass, in addition, the benzyl trimethylammonium chloride which is a cationic surfactant is stirred for further 1 hour so that it may become 10 g/l,

and non-dissolved cell residue is condensed to it — making — this — a filter paper (made in the Kiriyama factory, No.4) — using — Kiriyama — attraction filtration was carried out with the funnel and separation clearance of the condensation biomass residue was carried out. It was able to filter without carrying out blinding at this time. Stirring, in the obtained filtrate, the methanol was added, the crystal of 2 component copolymer of 3HB and 3HH(s) was deposited, this crystal was brought together by filtration in it, and it dried under reduced pressure to it. It was 96% when the recovery of 2 component copolymer of the obtained 3HB and 3HH(s) was calculated.
[0035] (Example 1 of a comparison) In the example 1, same actuation was performed except having not added benzyl trimethylammonium chloride. Blinding was not able to separate biomass residue violently in the phase of filtration.
[0036] (Example 2 of a comparison) In the example 8, same actuation was performed except having not added benzyl trimethylammonium chloride. Consequently, blinding was not able to separate biomass residue violently in the phase of filtration.
[0037]
[Effect of the Invention] Since according to this invention it becomes possible to make non-dissolved cell residue condense and to remove and PHA of a high grade is easily obtained by very simple actuation of adding the metal salt and surfactant more than divalent to the suspension of the microorganism biomass and extracting solvent containing PHA, this invention contributes to the improvement in effectiveness of industrial production of PHA by the microorganism, and reduction of cost greatly.

[Translation done.]